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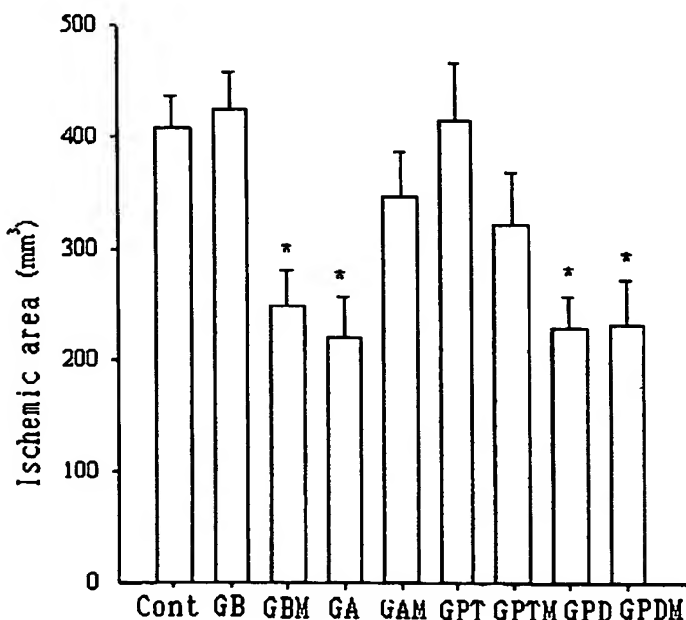
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(54) Title: NOVEL USE OF THE EXTRACT OF PROCESSED GINSENG AND SAPONIN ISOLATED THEREFROM



(57) Abstract: The present invention relates to novel use of the extract of processed ginseng extract having brain stroke protecting or treating activity. More particularly, the present invention relates to a processed ginseng extract with enhanced pharmacological effects due to subsequent treatment i.e., acid-treatment of ginseng and bio-converting treatment such as lactic acid bacterial fermenting and intestinal bacterial fermenting process. The processed ginseng extract in the present invention has preventing or treating effect for brain stroke. Therefore, it is useful in the prevention or treatment of brain stroke and brain diseases in human or mammal.

## NOVEL USE OF THE EXTRACT OF PROCESSED GINSENG AND SAPONIN ISOLATED THEREFROM

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### BACKGROUND OF THE INVENTION

#### Technical Field

10 The present invention relates to novel use of processed ginseng extract and the saponin compounds isolated therefrom for preventing and treating brain stroke and brain diseases in human or mammal. More particularly, the present invention relates to novel use of processed ginseng product with enhanced pharmacological effects due to serial treatment i.e., acid-treatment and subsequent bio-converting treatment such as lactic fermenting and intestinal-bacterial fermenting process.

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#### Background Art

Brain stroke is consisted of two type, i.e., ischemic stroke occurred from ischemic condition of brain tissue caused by intervention or decrease of blood supply to brain, and hemorrhagic stroke occurred from the bleeding of brain blood vessel where the former occupy about 80% among total patient suffered from brain stroke.

20 It has been reported that the cause of damage of brain neuronal cells are the release of excessive excitational neuronal transmitter, the production of free radical, the inhibition of protein synthesis, abnormal expression of gene and the activation of immune response etc., however, there has been not yet developed therapeutically effective agent to protect the damage of brain neuronal cells.

The inhibition of cyclooxygenase-2 (COX-2) results in protecting activity of brain neuronal cell due to the inhibition of glutamate release caused by inhibiting the reproduction of PGE<sub>2</sub>. Therefore, since many patients suffered from rheumatic disease and pains already have taken COX-2 inhibitor, much interest has been focused to the result of their clinical investigation about the co-relation between the incidence rate of brain stroke and the population of patient taken the drug, which may be new target for investigating effective agents to prevent or treat brain stroke (Iadecola, C. et al., *PNAS*, 30, pp1294-1299, 2001).

35 It is known that there are many genus of *Panax* genus plants belonged to Araliaceae, for example, *Panax ginseng* distributed or cultivated in far-eastern Asia region, *Panax quinquefolia* in America and Canada, *Panax notoginseng* in China, *Panax trifolia* in

eastern region of north America, *Panax japonica* in Japan, China and Nepal, *Panax pseudoginseng* in Nepal, *Panax vietnamensis* in Vietnam, *Panax elegator*, *Panax wangianus* and *Panax bipinratifidus* etc.

5       Hitherto, a ginseng has been widely known as a representative nutritive tonic agent. Recently, various scientific studies on the chemical constituents and pharmacological effects of the ginseng have been reported so that the secret pharmacological effects are paid attention with modern scientific approaches. Until now, it has been known that the ginseng has various pharmacological effects such as prevention of aging, anti-  
10       arteriosclerosis, treatment of hyperlipidemia, treatment of hepatic insufficiency, improvement of liver function, protection of radiation injury, immune enhancement, improvement of cerebral function, anti-thrombotic, anti-stress, anti-diabetic, anti-hypertensive, anti-tumor effects, etc.

15       It has been known that the main constituent of *Panax* genus plant is dammarane-skeleton type saponin. Ginsenosides Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Rg<sub>1</sub> and Re are the main saponins in *Panax ginseng*. Their biological activities are different from each other in accordance with their chemical structures.

20       There have been many attempts to modify the structure of the saponins to increase their pharmacological potency through processing.

25       Korean Patent Publication No. 10-1997-000239 issued on Jan. 21, 1997, discloses a process for preparing a processed ginseng prepared by subjecting hot temperature treatment containing high contents of ginsenoside Rg<sub>3</sub> and Rg<sub>5</sub> so as to obtaining processed ginseng having improved potency differing from original form of ginseng.

30       Korean Patent Publication No. 10-1997-061909 issued on Sep. 12, 1997, discloses a process for the production of saponin metabolites such as compound K from ginseng saponins using intestinal-bacteria.

35       However, there have been no disclosure or suggestion about a process for preparing processed *Panax* genus plant prepared by serial treatment comprising acid treatment and subsequent fermentation treatment with lactic-acid bacteria or intestinal-bacteria

40       The inventors of the present invention have intensively carried out the scientific investigation concerning chemical constituents and pharmacological effects of a ginseng, in particular a processing method of a ginseng and physiological activity of

the processed ginseng. As a result of the investigation, the inventors have discovered that through the serial treatment comprising acid treating and subsequent fermentation treating ginseng extract with lactic-acid bacteria or intestinal-bacteria, the extract of processed ginseng extract shows substantially enhanced pharmacological effects, especially, their preventing or treating activity for brain stroke and they have finally completed the present invention.

### SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a use of processed ginseng extract obtained by the steps essentially comprising acid treating ginseng extract and subsequent fermentation treating with lactic-acid bacteria or intestinal-bacteria and the saponin compounds isolated therefrom, in the manufacture of a medicament for the prevention or treatment of brain stroke disease.

And, another object of the present invention is to provide a method of treating or preventing brain stroke disease in a mammal comprising administering to said mammal an effective amount of above extract and the saponin compounds isolated therefrom, together with a pharmaceutically acceptable carrier thereof.

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### Disclosure of the invention

In accordance with the present invention, the present invention provides a pharmaceutical composition comprising processed ginseng extract obtained by the steps essentially comprising acid treating ginseng extract and subsequent fermentation treating with lactic-acid bacteria or intestinal-bacteria, as an active ingredient in an amount effective to treat or prevent human or mammal suffering from brain stroke and brain diseases, together with a pharmaceutically acceptable carrier.

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The present invention also provides a use of ginseng extract obtained by the steps essentially comprising acid treating ginseng extract and subsequent fermentation treating with lactic-acid bacteria or intestinal-bacteria in the preparation of the medicament to prevent or treat brain stroke and brain disease.

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Additionally, the present invention also provide a method of treating or preventing brain stroke disease in a mammal comprising administering to said mammal an

effective amount of above described extract, together with a pharmaceutically acceptable carrier thereof.

Above described extract can be prepared by following steps:

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1. 1<sup>st</sup> step:

1<sup>st</sup> step is to subject following acid treatment step to plant material as follows;

(1) Acid treatment step

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Specifically, at the 1<sup>st</sup> step, dried plant material of *Panax* genus, for examples, the root of *Panax ginseng*, is subjected to following acid treatment; for example, about 1 to 50 times, preferably 5 to 20 times of 0.01 to 50%, preferably, 0.1 to 10% acidic component, preferably, acetic acid, citric acid, lactic acid or acid-containing food such as the fruit of *Schizandra chinensis*, is added to the plant material and then is subjected to incubation at

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a temperature ranging from 20 to 80 °C, preferably 40 to 70 °C for a period ranging from 1 to 48 hrs, preferably, 3 to 12 hrs. Organic solvent such as methanol, ethanol, propanol, butanol, ether and ethyl acetate, is added thereto and then is subjected to extraction to obtain organic solvent soluble extract; the extract is neutralized with base finally to obtain the extract of chemically processed ginseng extract.

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The ginseng thus processed may be dried at a lower temperature than the heating temperature of the processing procedure, i.e., a normal temperature to 80 °C by a known manner to obtain a dried processed ginseng, or it may be further processed to obtain a powdered ginseng, if necessary.

25

Alternatively, the processed ginseng may be extracted using a known manner to obtain a processed ginseng extract. Specifically, the processed ginseng is extracted by using a solvent, and then the solvent is removed *in vacuo* or in freeze-drier to obtain a processed ginseng extract as dried powders.

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The solvent which may be employed herein includes a water, lower alcohol such as methanol, ethanol, etc., lower ketone such as acetone, methylethylketone, etc., supercritical fluid or mixed solvent thereof.

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The plant material which may be employed includes, but are limited to, *Panax* genus plant itself such as a fresh ginseng, a white ginseng and red ginseng, a fine root of ginseng or ginseng leaves or extracts thereof, which can be used as it is, finely divided

or powdered, processed product thereof and their by-product which comprise dammarane type saponin, preferably, the root, stem, petal, leaf, fruit of *Panax ginseng*, *Panax quinquefolia*, *Panax notoginseng*, *Panax trifolia*, *Panax japonica*, *Panax pseudoginseng*, *Panax vietnamensis*, *Panax elegator*, *Panax wangianus*, *Panax bipinratifidus* and *Panax angustifolium* and their tissue cultivates and the extract thereof.

Above (1) process can be subjected to plant material prior to following 2<sup>nd</sup> step.

## 2. 2<sup>nd</sup> step: fermentation step

The extract obtained from 1<sup>st</sup> step is subsequently subject to following bioconversion process such as fermentation with lactic acid or intestinal-bacteria as follows:

For example, lactic acid bacteria or intestinal-bacteria is added to the extract obtained from 1<sup>st</sup> step and incubated at a temperature ranging from 20 to 50 °C, preferably, 25 to 40 °C for a period ranging from 8 hours to 8 days, preferably 24 hours to 3 days to obtain extract fermented with bacteria.

The incubation time varies depending on the genus of used bacteria.

The lactic acid bacteria which may be employed includes any one which can metabolize ginsenoside Rg<sub>3</sub> to ginsenoside Rh<sub>2</sub>, preferably, lactic acid bacteria belonged to *Bifidobacterium* genus, more preferably, at least one or the mixture thereof selected from the group consisting of *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Lactobacillus lactis*, *Clostridium butyricum*, *Bifidobacterium* K-103, *Bifidobacterium* K-506, *Bifidobacterium* K-513, *Bifidobacterium* K-525, *Bifidobacterium* KK-1 and *Bifidobacterium* KK-2 (disclosed in *Arch. Pharm. Res.*, 21, p54-61, 1988).

The intestinal bacteria which may be employed includes any one which can metabolize ginsenoside Rg<sub>3</sub> to ginsenoside Rh<sub>2</sub>, preferably, intestinal-bacteria belonging to *Bacterioides*, *Fusobacterium* and *Eubacterium* genus, more preferably, at least one or the mixture thereof selected from the group consisting of *Bacterioides JY-6* (disclosed in *Biol. Pharm. Bull.*, 23, pp1481-1485, 2000), *Bacterioides stercoris*, *Fusobacterium* K-60 (disclosed in *Biol. Pharm. Bull.*, bid.) and *Eubacterium* L-8 (disclosed in *Biol. Pharm. Bull.*, bid.).

Further to above described steps, to isolate the saponin fractions or the saponin compounds from the extract obtained from above 2<sup>nd</sup> step, following process can be adopted.

### 3. 3<sup>rd</sup> step: Isolation process

To isolate pharmacologically active fractions or saponin compounds from the extract prepared by 2nd step, water, lower alcohols such as methanol, ethanol, propanol, butanol, ethylacetate, dichloromethane, chloroform, hexane, ether, or the mixed solvent thereof  
5 can be used to extract or isolate the fractions or compounds from the extract obtained from 2<sup>nd</sup> step as an appropriate solvent.

Additionally, the active ingredient can be extracted or isolated by subjecting special  
10 extraction method such as supercritical fluid extraction (SFE) to obtain partially purified saponin fractions and further, silica gel column chromatographic method to isolate individual saponins thereby.

Subsequent to above step, following processes such as drying process by lyophilization,  
15 agitation or dilution process can be adopted in addition to the above steps, if necessary.

Following processes can be selected either or both according to the final product forms of the present invention.

### 20 4. 4<sup>th</sup> step: Drying process

(1) Above ginseng extract obtained in Step 2 or 3, is concentrated *in vacuo* and then dried by lyophilization or spray drying.

(2) Above ginseng extract obtained in Step 2 or 3, is centrifuged to remove its impurities  
25 and precipitate and the supernatant is concentrated *in vacuo* and then dried by lyophilization or spray drying.

Through above 1<sup>st</sup> step to 2<sup>nd</sup> step processes, saponins such as ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc etc contained in plant material is transformed into chemically modified ginsenosides such  
30 as ginsenoside Rg<sub>3</sub> due to acid treatment or heat treatment in step 1 and then the sugar moiety at the position 3 in modified saponins is further degraded to form further modified saponins comprising degraded saponin ginsenoside Rh<sub>2</sub>.

The present invention also provides a pharmaceutical composition comprising  
35 saponin compounds selected from the group consisting of ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rf, Rg<sub>1</sub>, 20-ginsenoside Rg<sub>3</sub> and the mixture thereof, preferably, 20-ginsenoside Rg<sub>3</sub> as an active ingredient in an amount effective to treat or prevent

human or mammal suffering from brain diseases, together with a pharmaceutically acceptable carrier.

The present invention also provides a use of saponin compounds selected from the group consisting of ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rf, Rg<sub>1</sub>, 20-ginsenoside Rg<sub>3</sub> and the mixture thereof, preferably, 20-ginsenoside Rg<sub>3</sub> in the preparation of the medicament to prevent or treat brain stroke and brain diseases.

Additionally, the present invention also provide a method for treating or preventing brain stroke and brain diseases in a mammal comprising administering to said mammal an effective amount of saponin compounds selected from the group consisting of ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rf, Rg<sub>1</sub>, 20-ginsenoside Rg<sub>3</sub> and the mixture thereof, preferably, 20-ginsenoside Rg<sub>3</sub>, together with a pharmaceutically acceptable carrier thereof.

The inventive composition may additionally comprise conventional carrier, adjuvants or diluents in accordance with a using method. It is preferable that said carrier is used as appropriate substance according to the usage and application method, but it is not limited. Appropriate diluents are listed in the written text of Remington's Pharmaceutical Science (Mack Publishing co, Easton PA).

Hereinafter, the following formulation methods and excipients are merely exemplary and in no way limit the invention.

The composition according to the present invention can be provided as a pharmaceutical composition containing pharmaceutically acceptable carriers, adjuvants or diluents, e.g., lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starches, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, polyvinyl pyrrolidone, water, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate and mineral oil. The formulations may additionally include fillers, anti-agglutinating agents, lubricating agents, wetting agents, flavoring agents, emulsifiers, preservatives and the like. The compositions of the present invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after their administration to a patient by employing any of the procedures well known in the art.

For example, the compositions of the present invention can be dissolved in oils, propylene glycol or other solvents that are commonly used to produce an injection.



Suitable examples of the carriers include physiological saline, polyethylene glycol, ethanol, vegetable oils, isopropyl myristate, etc., but are not limited to them. For topical administration, the compounds of the present invention can be formulated in the form of ointments and creams.

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Pharmaceutical formulations containing present composition may be prepared in any form, such as oral dosage form (powder, tablet, capsule, soft capsule, aqueous medicine, syrup, elixirs pill, powder, sachet, granule), or topical preparation (cream, ointment, lotion, gel, balm, patch, paste, spray solution, aerosol and the like), or injectable preparation (solution, suspension, emulsion).

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The composition of the present invention in pharmaceutical dosage forms may be used in the form of their pharmaceutically acceptable salts, and also may be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds.

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The desirable dose of the inventive extract or compounds of the present invention varies depending on the condition and the weight of the subject, severity, drug form, route and period of administration, and may be chosen by those skilled in the art. However, in order to obtain desirable effects, it is generally recommended to administer at the amount ranging 0.01-10g/kg, preferably, 1 to 5g/kg by weight/day of the inventive extract or compounds of the present invention. The dose may be administered in single or divided into several times per day. In terms of composition, the complex herbal composition should be present between 0.01 to 80% by weight, preferably 0.5 to 50% by weight based on the total weight of the composition.

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The pharmaceutical composition of present invention can be administered to a subject animal such as mammals (rat, mouse, domestic animals or human) via various routes. All modes of administration are contemplated, for example, administration can be made orally, rectally or by intravenous, intramuscular, subcutaneous, intracutaneous, intrathecal, epidural or intracerebroventricular injection.

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The present inventors demonstrated that present composition comprising above described ginseng extract or compounds of the present invention have preventing or treating activity of brain stroke by accomplishing *in vivo* experiment already well known in the art, e.g., middle cerebral artery occlusion model test which is consisted of following step i.e., nylon filament is inserted into internal carotid artery to occlude

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middle cerebral artery and 120 minutes after, the filament is removed again to allow the reperfusion of the artery.

Accordingly, it is another object of the present invention to provide a health care  
5 food comprising above described extract or compounds of the present invention prepared by above processes and a sitologically acceptable additive to prevent brain stroke and brain diseases.

Above described composition therein can be added to food, additive or beverage for  
10 prevention of brain stroke diseases. For the purpose of preventing brain stroke diseases, wherein, the amount of above described extract or compounds of the present invention in food or beverage may generally range from about 0.1 to 15 w/w %, preferably 1 to 10 w/w % of total weight of food for the health food composition and 1 to 30 g, preferably 3 to 10 g on the ratio of 100ml of the health beverage composition.

15 Providing that the health beverage composition of present invention contains above described extract or compounds of the present invention as an essential component in the indicated ratio, there is no particular limitation on the other liquid component, wherein the other component can be various deodorant or natural carbohydrate etc such  
20 as conventional beverage. Examples of aforementioned natural carbohydrate are monosaccharide such as glucose, fructose etc; disaccharide such as maltose, sucrose etc; conventional sugar such as dextrin, cyclodextrin; and sugar alcohol such as xylitol, and erythritol etc. As the other deodorant than aforementioned ones, natural deodorant such as  
25 taumatin, stevia extract such as levaudioside A, glycyrrhizin et al., and synthetic deodorant such as saccharin, aspartam et al., may be useful favorably. The amount of above described natural carbohydrate is generally ranges from about 1 to 20 g, preferably 5 to 12 g in the ratio of 100ml of present beverage composition.

The other components than aforementioned composition are various nutrients, a  
30 vitamin, a mineral or an electrolyte, synthetic flavoring agent, a coloring agent and improving agent in case of cheese chocolate et al., pectic acid and the salt thereof, alginic acid and the salt thereof, organic acid, protective colloidal adhesive, pH controlling agent, stabilizer, a preservative, glycerin, alcohol, carbonizing agent used in carbonate beverage et al. The other component than aforementioned ones may be fruit juice for preparing  
35 natural fruit juice, fruit juice beverage and vegetable beverage, wherein the component can be used independently or in combination. The ratio of the components is not so important but is generally range from about 0 to 20 w/w % per 100 w/w % present

composition.

Examples of addable food comprising aforementioned extract therein or compounds of the present invention are various food, beverage, gum, vitamin complex, health improving food and the like.

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It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, use and preparations of the present invention without departing from the spirit or scope of the invention.

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### **Brief Description of the Drawings**

The above and other objects, features and other advantages of the present invention will more clearly understood from the following detailed description taken in conjunction with the accompanying drawing, in which;

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Fig. 1 Shows the effect of an processed ginseng extract and saponin compounds thereinfrom.

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### **Best Mode for Carrying Out the Invention**

The present invention is more specifically explained by the following examples. However, it should be understood that the present invention is not limited to these examples in any manner.

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#### **Example 1. Preparation of processed ginseng extract (1)**

500g of six-year *Panax ginseng* root procured from Kyung Dong Market, was sliced into pieces, extracted with 5 L of methanol at five times and concentrated to obtain 25g of non-processed ginseng extract. The extract was dissolved in 30 ml of distilled water, extracted with 1500 ml of butanol at four times and concentrated to obtain 10g of butanol fraction. Each 10 g of *Bifidobacterium* KK-1 (Accession number of Depository Authority: KCCM 10364) and 10 g of *Bifidobacterium* KK-2 (Accession number of Depository Authority: KCCM 10365) were added thereto and then was incubated at 37°C for 72 hours to obtain incubate thereof. The incubates were extracted with butanol, concentrated and dried to obtain 4 g of processed ginseng extract designated as GBM thereafter.

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**Example 2. Preparation of processed ginseng extract (2)**

1 kg of six-year *Panax ginseng* root was sliced into pieces, extracted with 10 L of methanol at five times and concentrated to obtain 50g of ginseng extract. The extract  
5 was dissolved in 50 ml of distilled water, extracted with 3000 ml of butanol at four times and concentrated to obtain 20g of butanol fraction designated as GB thereafter.

**Example 3. Preparation of processed ginseng extract (3)**

10g of six-year *Panax ginseng* root were sliced into pieces. 1 L of distilled water containing 0.1% lactic acid was added thereto and incubated at 60°C for 5 hours. The pH of cultivates was adjusted to 6.8-7.0 and extracted with 3 L of butanol at three times to obtain 6.5g of processed ginseng extract designated as GA thereafter.

**Example 4. Preparation of processed ginseng extract (4)**

10g of six-year *Panax ginseng* root were sliced into pieces. 1 L of distilled water containing 0.1% lactic acid was added thereto and incubated at 60°C for 5 hours. The pH of cultivates was adjusted to 6.8-7.0 and extracted with 3 L of butanol at three  
20 times to obtain 6.5g of processed ginseng extract. Each 5 g (wet weight) of *Bifidobacterium* KK-1 (Accession number of Depository Authority: KCCM 10364) and *Bifidobacterium* KK-2 (Accession number of Depository Authority: KCCM 10365) were added thereto and then was incubated at 37°C for 72 hours to obtain incubates thereof. The incubates were extracted with butanol, concentrated and dried to  
25 obtain 3.5 g of processed ginseng extract designated as GAM thereafter.

**Example 5. Preparation of processed ginseng extract (5)**

1 kg of sliced *Panax ginseng* was extracted with 10 L of methanol at five times and concentrated *in vacuo* to obtain 50g of the extract. The extract was dissolved in 50 ml  
30 of distilled water, extracted with 3000 ml of butanol at four times and concentrated to obtain 20g of butanol fraction. The fraction was further subjected to Silica gel column chromatography (Column size: 3.5x60 cm, Developing Solvent System: chloroform: MeOH = 10:1) to isolate 2g of saponin fraction containing abundant amount of  
35 ginsenoside Re, Rf and Rg1, designated as GPT thereafter.

**Example 6. Preparation of processed ginseng extract (6)**

1 kg of sliced *Panax ginseng* was extracted with 10 L of methanol at five times and concentrated *in vacuo* to obtain 50g of the extract. The extract was dissolved in 50 ml of distilled water, extracted with 3000 ml of butanol at four times and concentrated to obtain 20g of butanol fraction. The fraction was further subjected to Silica gel column chromatography (Column size: 3.5x60 cm, Developing Solvent System: chloroform: MeOH = 10:1) to isolate 2g of saponin fraction containing abundant amount of ginsenoside Re, Rf and Rg. Each 3 g (wet weight) of *Bifidobacterium* KK-1 (Accession number of Depository Authority: KCCM 10364) and *Bifidobacterium* KK-2 (Accession number of Depository Authority: KCCM 10365) were added thereto and then was incubated at 37°C for 72 hours to obtain incubates thereof. The incubates were extracted with butanol, concentrated and dried to obtain 1.2 g of processed ginseng extract designated as GPTM thereafter.

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**Example 7. Preparation of processed ginseng extract (7)**

1 kg of sliced six-year *Panax ginseng* was extracted with 10 L of methanol at five times and concentrated *in vacuo* to obtain 50g of the extract. The extract was dissolved in 50 ml of distilled water, extracted with 3000 ml of butanol at four times and concentrated to obtain 20g of butanol fraction. The fraction was further subjected to Silica gel column chromatography (Column size: 3.5x60 cm, Developing Solvent System: chloroform: MeOH = 10:1) to isolate 2.5g of saponin fraction containing abundant amount of ginsenoside Rb1, Rb2, Rc and Rd, designated as GPD thereafter.

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**Example 8. Preparation of processed ginseng extract (8)**

1 kg of sliced six-year *Panax ginseng* was extracted with 10 L of methanol at five times and concentrated *in vacuo* to obtain 50g of the extract. The extract was dissolved in 50 ml of distilled water, extracted with 3000 ml of butanol at four times and concentrated to obtain 20g of butanol fraction. The fraction was further subjected to Silica gel column chromatography (Column size: 3.5x60 cm, Developing Solvent System: chloroform: MeOH = 10:1) to isolate 2.5g of saponin fraction containing abundant amount of ginsenoside Rb1, Rb2, Rc and Rd. Each 3 g (wet weight) of *Bifidobacterium* KK-1 (Accession number of Depository Authority: KCCM 10364) and *Bifidobacterium* KK-2 (Accession number of Depository Authority: KCCM 10365) were added thereto and then was incubated at 37°C for 72 hours to obtain

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incubates thereof. The incubates were extracted with butanol, concentrated and dried to obtain 2 g of processed ginseng extract designated as GPDM thereafter.

#### Comparative Example 1. Preparation of non-processed ginseng extract

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20g of sliced five-year *Panax ginseng* was extracted with five times of distilled water at 60°C for 5 hours, concentrated *in vacuo* with evaporator (Eyella, KN-IN model, Japan) and dried by lyophilization (Samwon Nangyul Co. SFDSM24L Model, Korea) to obtain 1g of non-processed ginseng powder.

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#### Comparative Example 2. Preparation of acid treated ginseng extract

20g of five-year *Panax ginseng* root was sliced into pieces. 2000 ml of distilled water containing 0.1% lactic acid was added thereto and incubated at 60°C for 5 hours. 5000 ml of butanol was added thereto, extracted, concentrated *in vacuo* with evaporator (Eyella, KN-IN model, Japan) and dried by lyophilization (Samwon Nangyul Co. SFDSM24L Model, Korea) to obtain 1.5g of acid treated ginseng extract.

#### 20 Experimental Example 1: Content Analysis Experiment

Each 2g of extract obtained from above Comparative Example 1, 2 and Example 1 and 5 were extracted with 100 ml of methanol at three times. The methanol soluble layer was concentrated *in vacuo* and suspended with 100 ml of distilled water. The suspension was extracted with 100 ml of ether solvent at three times, concentrated *in vacuo*. And further, the concentrates was extracted with 100 ml of butanol at three times and concentrated *in vacuo* to obtain their concentrates. The concentrates were dissolved in 100ml of MeOH to obtain 100 mg of saponin fraction. The content analysis was subjected TLC(solvent system : chloroform:methanol:water=65:35:10, spraying reagent : 5% methanolic sulfuric acid solution) and TLC scanner (Shimadzu, CS-9301PC) as a detector. The results thus obtained are shown in Table 1 below

Table 1: The saponin amount of Comparative Example (CE) 1, 2 and Example (E) 1, 4

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Component	The saponin amount among total saponin fraction (%)			
	CE 1	CE 2	E 1	E 4

Ginsenoside Rb1	15.1	2.5	<1	<1
Ginsenoside Rb2	8.2	2	<1	<1
Ginsenoside Rc	9.5	1.8	<1	<1
Ginsenoside Rd	3.5	<1	4.5	<1
Ginsenoside Rg3	<1	25	<1	9
Ginsenoside F2	<1	<1	5.5	0.9
Compound K	<1	<1	16.1	1.5
Ginsenoside Rh2	<1	<1	<1	14
Protopanaxadiol	<1	<1	<1	1.5

As a result, while the content of ginsenoside F2 and compound K was significantly increased in Example 1 treated with lactic acid bacteria, the content of ginsenoside Rg3, Rh2 and protopanaxadiol was significantly increased in Example 4 treated with acid and subsequent lactic acid bacteria.

#### **Experimental Example 2: preventing or treating activity for Brain stroke**

In order to confirm the preventing or treating activity of processed ginseng extract and saponin compounds isolated therefrom for Brain stroke and to compare the activity of present invention with those of non-processed ginseng extract in Comparative Example 1 and acid treated ginseng extract in Comparative Example 2, the experiment was performed by following procedure.

#### **Method**

At about five minutes before reperfusion, various concentrations of test samples were administrated to ischemic brain animal model. And then, re-perfusion was started. At 24 hours after 1<sup>st</sup> operation, the animal was killed to deliver its brain which was further cut into brain slice at the width of 2 mm using by brain matrix and stained with 2,3,5-triphenyltetrazolium chloride (TTC) staining method. The cerebral infarction region was analyzed by image analysis system.

#### **Result**

As shown in Fig. 1, the processed ginseng extract of Example 1(GBM), saponin fraction of Example 3 (GA), saponin compounds in Example 7 (GPD) and Example 8 (GPDM) showed significant protecting activity for brain neuronal cells and it is confirmed that their activities were superior to Ebselin® (Sigma co.) and baicalein

used as positive control. Control group treated with vehicle only is designated as Cont, Example 1 as GBM, Example 2 as GB, Example 3 as GA, Example 4 as GAM, Example 5 as GPT, Example 6 as GPTM, Example 7 as GPD, Example 8 as GPDm. “\*” denotes that the value has significant at 95% significant level compared with control group.

As described above, it is confirmed that processed ginseng extract prepared by the present invention shows therapeutic and protective effect for brain stroke and thus, it is useful for anti-brain stroke drug or health care food.

### Experimental Example 3. Toxicity test

#### Methods (1)

The acute toxicity tests on ICR mice (mean body weight  $25 \pm 5$ g) and Sprague-Dawley rats ( $235 \pm 10$ g, Hyochang Science) were performed using the extract of the Example 1. Four group consisting of 10 mice or rats was administrated orally intraperitoneally with 500mg/kg, 725mg/kg, 1000mg/kg and 5000mg/kg of test sample or solvents (0.2 ml, i.p.) respectively and observed for 2 weeks.

#### Methods (2)

The acute toxicity tests on ICR mice and Sprague-Dawley rats were performed using the extract of the Example 1. Four group consisting of 10 mice or rats was administrated intraperitoneally with 25mg/kg, 250mg/kg, 500mg/kg and 725mg/kg of test sample or solvents (0.2 ml, i.p.), respectively and observed for 24 hours.

#### Results

There were no treatment-related effects on mortality, clinical signs, body weight changes and gross findings in any group or either gender. These results suggested that the extract prepared in the present invention were potent and safe.

Hereinafter, the formulating methods and kinds of excipients will be described, but the present invention is not limited to them. The representative preparation examples were described as follows.

#### Preparation of powder

Dried powder of Example 1	50mg
Lactose	100mg
Talc	10mg



Powder preparation was prepared by mixing above components and filling sealed package.

Preparation of tablet

5	Dried powder of Example 1	50mg
	Corn Starch	100mg
	Lactose	100mg
	Magnesium Stearate	2mg
	Tablet preparation was prepared by mixing above components and entabletting.	

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Preparation of capsule

	Dried powder of Example 1	50mg
	Corn starch	100mg
	Lactose	100mg
15	Magnesium Stearate	2mg
	Tablet preparation was prepared by mixing above components and filling gelatin capsule by conventional gelatin preparation method.	

Preparation of injection

20	Dried powder of Example 1	50mg
	Distilled water for injection	optimum amount
	PH controller	optimum amount
	Injection preparation was prepared by dissolving active component, controlling pH to about 7.5 and then filling all the components in 2ml ample and sterilizing by	
25	conventional injection preparation method.	

Preparation of liquid

	Dried powder of Example 1	0.1~80g
	Sugar	5~10g
30	Citric acid	0.05~0.3%
	Caramel	0.005~0.02%
	Vitamin C	0.1~1%
	Distilled water	79~94%
	CO <sub>2</sub> gas	0.5~0.82%
35	Liquid preparation was prepared by dissolving active component, filling all the components and sterilizing by conventional liquid preparation method.	

Preparation of health care food

	Extract of Example 1	1000mg
	Vitamin mixture	optimum amount
	Vitamin A acetate	70µg
5	Vitamin E	1.0mg
	Vitamin B <sub>1</sub>	0.13mg
	Vitamin B <sub>2</sub>	0.15mg
	Vitamin B <sub>6</sub>	0.5mg
	Vitamin B <sub>12</sub>	0.2µg
10	Vitamin C	10mg
	Biotin	10µg
	Amide nicotinic acid	1.7mg
	Folic acid	50µg
	Calcium pantothenic acid	0.5mg
15	Mineral mixture	optimum amount
	Ferrous sulfate	1.75mg
	Zinc oxide	0.82mg
	Magnesium carbonate	25.3mg
	Monopotassium phosphate	15mg
20	Dicalcium phosphate	55mg
	Potassium citrate	90mg
	Calcium carbonate	100mg
	Magnesium chloride	24.8mg
25	The above-mentioned vitamin and mineral mixture may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention.	

Preparation of health beverage

	Extract of Example 1	1000mg
30	Citric acid	1000mg
	Oligosaccharide	100g
	Apricot concentration	2g
	Taurine	1g
	Distilled water	900ml
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Health beverage preparation was prepared by dissolving active component, mixing, stirred at 85 °C for 1 hour, filtered and then filling all the components in 1000ml

ample and sterilizing by conventional health beverage preparation method.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

#### **Industrial Applicability**

10 The composition comprising the processed ginseng extract treated with acid and subsequent fermentation by biological treatment with lactic acid bacterial or intestinal-bacterial culture according to the present invention, shows preventing or treating effect for Brain stroke. Therefore, it is useful in the prevention or treatment of brain stroke in human or mammal.

## CLAIMS

1. A pharmaceutical composition comprising processed ginseng extract obtained by the steps essentially comprising acid treating ginseng extract and subsequent  
5 fermentation treating with lactic-acid bacteria or intestinal-bacteria, as an active ingredient in an amount effective to treat or prevent human or mammal suffering from brain strokes and brain diseases, together with a pharmaceutically acceptable carrier.
2. The pharmaceutical composition effective for treating or preventing human or  
10 mammal suffering from brain strokes and brain diseases according to claim 1, wherein said plant material comprises the root, stem, petal, leaf, fruit and their tissue cultivates thereof.
3. The pharmaceutical composition for treating or preventing human or mammal  
15 suffering from brain strokes and brain diseases according to claim 1, wherein said acid is acetic acid, citric acid, lactic acid or acid-containing food.
4. The pharmaceutical composition for treating or preventing human or mammal  
20 suffering from brain strokes and brain diseases according to claim 1, wherein said lactic acid bacteria is belonged to *Bifidobacterium* genus or *Lactobacillus* genus bacteria.
5. The pharmaceutical composition according to claim 4 wherein said lactic acid  
25 bacteria comprises at least one or the mixture thereof selected from the group consisting of *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Lactobacillus lactis*, *Clostridium butyricum*, *Bifidobacterium* K-103, *Bifidobacterium* K-506, *Bifidobacterium* K-513, *Bifidobacterium* K-525, *Bifidobacterium* KK-1 and *Bifidobacterium* KK-2.
- 30 6. The pharmaceutical composition for treating or preventing human or mammal suffering from brain strokes and brain diseases according to claim 1, wherein said intestinal bacteria is belonged to *Bacteriodes* genus, *Fusobacterium* genus or *Eubacterium* genus bacteria.
- 35 7. The pharmaceutical composition according to claim 6 wherein intestinal-bacteria comprises at least one or the mixture thereof selected from the group consisting of *Bacteriodes* JY-6, *Bacteriodes stercoris*, *Fusobacterium* K-60 and *Eubacterium* L-8.

8. A pharmaceutical compositions comprising saponin compounds selected from the group consisting of ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, Rd, Re, Rf, Rg<sub>1</sub>, 20-ginsenoside Rg<sub>3</sub>, and the mixture thereof, as an active ingredient in an amount effective to treat or prevent human or mammal brain stroke and brain diseases, together with a pharmaceutically acceptable carrier.

9. The pharmaceutical composition according to any of claims 1 to 8, wherein said pharmaceutical composition is provided in an acceptable carrier as powder, granule, tablet, capsule, aqueous medicine or injection.

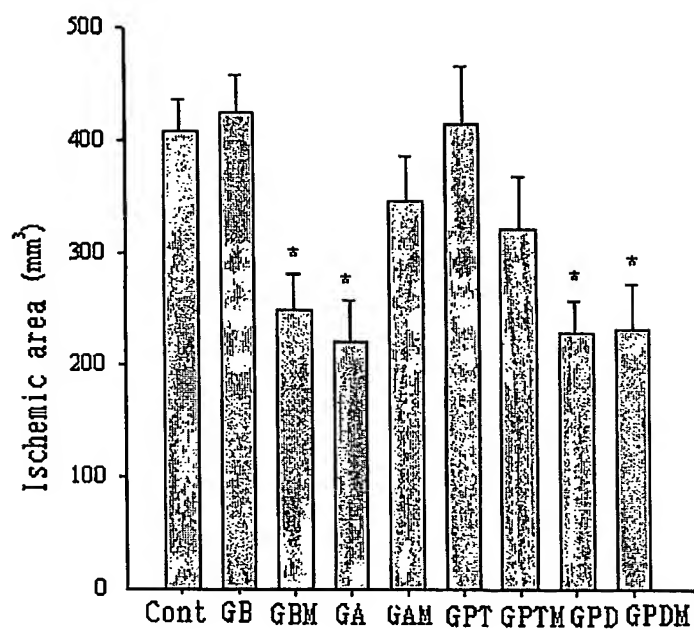
10. A use of ginseng extract obtained by the steps essentially comprising acid treating ginseng extract and subsequent fermentation treating with lactic-acid bacteria or intestinal-bacteria in the preparation of the medicament to prevent or treat brain stroke and brain disease.

11. A health care food comprising the extract as set forth in claim 1 and a sitologically acceptable additive to prevent cancer or allergic diseases.

12. The health care food according to claim 12, wherein said health care food is provided as beverage type.



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Fig.1



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/KR03/00704

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> <b>IPC7 A61K 35/78</b> According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) A61K 35/78, A61K 31/70, A61K 31/00 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched KOREAN PATENTS AND APPLICATIONS FOR INVENTIONS SINCE 1975 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubMed on line		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, Y	KR 2002-0084311 A (Digitalbiotech Co., Ltd.), 07 November 2002 See entire document	1-12
A	Hasegawa H et al. 'Main ginseng saponin metabolites formed by intestinal bacteria' In; Planta Med. 1996 Oct.; 62(5): 453-7	1-12
P, A	EP 1213026 A1 (Japan Science & Tech Corp.), 12 June 2002 See entire document	1-12
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.		
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation, or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>		
Date of the actual completion of the international search 30 JUNE 2003 (30.06.2003)		Date of mailing of the international search report 30 JUNE 2003 (30.06.2003)
Name and mailing address of the ISA/KR  Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140		Authorized officer YE0, Ho Sup Telephone No. 82-42-481-5627 

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2002-0084311	07/11/2002	NONE	
EP 1213026 A1	12/06/2002	WO 0115717 A1 JP 1139483 A2	08/03/2001 22/05/2001